

HISTORIES OF GROWTH AND CONDITION FROM TEETH OF HARBOR SEALS IN PRINCE WILLIAM SOUND AND SOUTHEAST ALASKA, 1995-1999

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INTRODUCTION

Pinnipeds are apex predators that integrate environmental conditions over broad spatial and temporal scales. Seasonal and inter-annual changes in the physical and biological conditions encountered by pinnipeds are likely to influence their behavior, physical condition, and life history parameters (e.g., growth rates, reproductive condition). Such ecological changes have been reported for Alaska fish stocks (e.g., Hollowed and Wooster 1992, Bakkala 1993, Francis and Hare 1994), characteristics of the region's physical environment (e.g., Salmon 1992, Schwing 1994, Trenberth and Hurrell 1994), and seabird and marine mammal populations (e.g., Roby and Brink 1986, Springer *et al.* 1986, Trites 1992).

Patterns of growth recorded in pinniped teeth have been widely used to estimate age by counting regularly deposited layers of dentine or cementum. In some cases, this method has been extended to provide estimates of the timing of life history events, such as maturation, parturition, or periods of extreme growth rates in individual seals' lives by measuring the widths of layers and making inference from variability in those widths (e.g., Bengtson and Laws 1985, Bengtson 1988, Baker 1991, Boyd and Roberts 1993). Because the physiological conditions that are recorded as changes in tooth growth rates are influenced by various sources of environmental variability, teeth can also provide a historical record of conditions experienced by an individual or population. For example, Boyd and Roberts (1993) found a strong correlation between tooth growth in male Antarctic fur seals (*Arctocephalus gazella*) and an index of the El Niño/Southern Oscillation, providing a measure of the sensitivity of Antarctic fur seals to broad-scale environmental variability.

This report summarizes the results of a collaborative study by the Alaska Department of Fish and Game (ADF&G) and the National Marine Fisheries Service (NMFS), National Marine Mammal Laboratory (NMML) to investigate the life history and growth of harbor seals in Alaska using patterns in the deposition of material in the seals' teeth. The first phase of the project, a study of the feasibility of estimating age at sexual maturation from transition zones in the cementum of harbor seal teeth, was reported by Baker and Boveng (1997). The second phase of the project involved developing and refining procedures for preparing and analyzing seal teeth, and determining whether teeth can be used to derive cohort- or year-specific histories of growth and condition. The specific objectives of the second phase were to refine techniques for preparing seal tooth sections that provide better resolution and accuracy in measuring annual growth increments; and to construct a model of tooth growth that could be used to detect annual and cohort-specific variations in growth

and condition of Alaska harbor seals.

METHODS

The ADF&G provided a canine tooth from each of 76 harbor seals collected in Southeast Alaska and Prince William Sound, 1995-1999, from the archives of the ADF&G and the University of Alaska Museum, Fairbanks. Post-canine teeth were also provided from 50 of these seals. The teeth of 72 harbor seals were aged and measured for use in our analyses. Fifty-seven teeth were from Southeast Alaska and 15 teeth from Prince William Sound. Prior to processing the harbor seal teeth, approximately 30 canine and post canine teeth from various species of pinnipeds were obtained from the NMML. These teeth, for which no supporting data (date of collection, sex, location, etc.) were available, were used to refine a method for cutting and mounting thin sections.

Cutting and mounting thin sections

Each tooth was either coated with or cast in a block of optical-grade epoxy resin (Epotek 401[®]). The tooth was cut longitudinally (medial-distal) with a petrographic trim saw, just off center so that the saw kerf was taken entirely from one half of the tooth. The cut face of the more complete half of the tooth was then polished on a Buehler Ecomet III[®] grinder with 600 grit abrasive paper. The polished face was glued to a glass slide using the optical epoxy. The portion of the tooth that was glued to the slide was cut and ground to a thickness of 0.12 mm using a Hillquist[®] thin-section machine. A glass coverslip was affixed over the tooth section using Permount[®] mounting medium.

We undertook tests using additional seal teeth that were available at the NMML to determine whether clarity and definition of cementum layers could be improved by cutting the tooth in a different plane (e.g., buccal-lingual rather than medial-distal), staining the tooth section (without decalcification), clearing the tooth, or a combination of these techniques. We ran a series of experiments using toluidine blue and xylene, varying the time the sample was immersed, the concentration of the stain and the method of application. We did not find that these techniques enhanced the appearance of growth layers and the techniques were not incorporated into our standard procedures.

Aging and estimation of ASM

The mounted tooth sections were examined under polarized light using a stereo dissecting microscope at magnifications of 4X – 32X. Two readers estimated total age independently for each tooth, examining both cementum and dentine. One opaque band and one translucent band were counted for each year of life. Decimal fractions of years were added to the counts of whole growth layers in consideration of the dates on which the seals were killed, assuming most harbor seal pups are born in June (Table 1). In most cases, fractional ages appeared consistent with the banding in the outermost, incomplete cementum layer. One reader also examined each tooth for a “transition zone” in the widths of cementum layers; such transitions have been used to estimate the age of sexual maturation in other species.

Image capture, measurement, and analysis

A system for capturing digital images from a dissecting microscope and recording measurements from the images was acquired and installed at the NMML. The system includes a Polaroid® digital camera, a stereo dissecting microscope (provided by the NMML), a desktop computer, and Media Cybernetics Optimas® image analysis software version 6.5. A macro was developed in Optimas to measure growth layers of dentine in standardized locations on the tooth sections and to export measurements to an Excel spreadsheet.

Although one of our objectives was to model the age-specific growth of cementum in harbor seals, we found that the cementum layering in these seals was too variable and indistinct to allow development of a standardized protocol for measuring layer widths. We therefore modified our objectives to focus solely on patterns in dentine growth. Because the pulp cavity begins to occlude after just a few years of life in harbor seals, we were limited in our measurements and analyses to the dentine layers deposited in the first 2 years of life.

We defined fetal dentine as the layer deposited in utero, “year-one” as the layer deposited between birth and age one, and “year-two” as the layer deposited between age one and age two. The widths of these layers of dentine in the canine teeth were measured using a procedure that we developed for standardizing the location and acquisition of these measurements. Growth layers were measured in four places on each canine tooth. Fetal dentine was measured at the end of the enamel on the crown of the tooth on both the anterior and posterior side (Fig. 1) of the tooth section. Annual dentine layers were measured on both the anterior and posterior sides of the tooth along a transect located at a standardized position at the midpoint of the overlap of the fetal dentine and cementum (Fig. 2).

RESULTS

An initial inspection of the prepared slides indicated that certain features, such as the neonatal line in the dentine could be seen clearly in most specimens. The appearance of growth layers in the cementum, however, was not substantially clearer than specimens prepared previously by decalcification and staining techniques (i.e., the specimens analyzed by Baker and Boveng (1997)). The post-canine teeth generally displayed clearer growth layers in the cementum, and the canine teeth generally displayed clearer growth layers in the dentine, though only the first 1–3 annual layers were typically distinct. Differences in the estimated age for canine teeth and estimated age for post canines from the same seal were small (Fig. 3). A linear regression through the points in Figure 3 resulted in a slope of 1.05 and an intercept of 0.12, not significantly different from a slope of 1.0 and intercept of 0 (line shown in Fig. 3).

The average age of seals from Southeast Alaska was 5.9 (n=57, SE 4.4, range 0.4 to 18.5). The average age of seals from Prince William Sound was 7.2 (n=15, SE 6.1, range 0.3 to 19.3). The age distributions of the seals (Fig. 4) appeared similar for Southeast Alaska and Prince William Sound, though the sample size from Prince William Sound was too small for statistical comparisons.

A transition zone, or age of sexual maturity (ASM), was estimated for 19 of the 72 samples, however the sex of seals from Prince William Sound were unknown so we could not test for differences between the sexes of the entire sample. Of the 57 teeth from Southeast Alaska, we were able to detect a transition zone in 4 females and 9 males. The average age of the transition zones were 5.5 years for the females and 5.1 years for the males, not significantly different. Of the 44

teeth from Southeast Alaska that did not exhibit a transition zone, 3 females and 8 males were older than the average ASM. The remaining teeth were from young animals, most of which had probably not matured.

There were positive correlations between widths of anterior and posterior dentine layers (e.g., Fig. 5, $r = 0.50$) and between year-one and year-two dentine layers (e.g., Fig. 6, $r = 0.66$). These correlations indicate that it may be important to account for the overall size of each tooth when testing for differences in layer widths among cohorts or populations from different regions or time periods. We attempted to do this when searching for cohort specific patterns in growth layer widths by using both raw growth layer widths and widths of the year-one and year-two layers scaled (divided) by the corresponding fetal dentine width. There were no obvious cohort-specific patterns in either the raw (Fig. 7) or scaled (Fig. 8) dentine layer widths. There may be more effective measures than fetal dentine width for scaling in this manner, such as total length of the tooth or length of the enamel crown. We began recording some of these measures later in the study, but did not have a complete enough record to apply them to our analysis.

There were no significant differences between Prince William Sound and Southeast Alaska in any of the widths of growth layers that we examined (Tables 2 and 3). An analysis of the sample sizes required, given the variability that we observed, to detect specified differences between two populations is shown in Table 4. Because there were so few seals from Prince William Sound in our sample, we would have been able to detect only relatively large differences in layer widths between seals from the two regions. It is apparent from Table 4, however, that samples of 100-200 seals would provide acceptable confidence of detecting differences of as small as 10% between populations from different regions or time periods, assuming that the means and variances that we observed are typical.

DISCUSSION

Refinement of tooth preparation techniques

The success of analyses of tooth growth layers, especially in cementum, is highly dependent on the quality of preparation of the tooth specimens. It is difficult to prepare adequate tooth sections with the saws and grinders that have typically been used for marine mammal teeth (Leiberman *et al.* 1990). A common alternative technique, decalcifying and staining, also has disadvantages such as potential damage to the outermost cementum layers and the requirement to remove the enamel crown, which we found to be helpful if not critical as a landmark for standardizing the locations of our measurements. The technique we used for preparation of teeth, resulting in glass-mounted thin sections, is labor intensive but it provides relatively permanent, high-quality slides. We were unable to improve the definition of growth layers by staining or clearing, but there may still be other modifications worth testing. For example, a combination of light etching or decalcification of the exposed surface of the tooth after the other surface has been mounted on a slide may allow greater quantities of stain to be absorbed by portions of the layers.

Using tooth growth to detect variations in growth and condition of Alaska harbor seals

We have concluded that cementum growth in Alaska harbor seals does not provide a record that is sufficiently clear to allow age-specific modeling of cementum growth for comparative

analyses among cohorts, regions, or time periods. Dentine layers deposited *in utero* and in the first and second years of life, however, may satisfy at least the statistical requirements for these analyses, given sufficient sample sizes. We do not yet know whether to expect that dentine layers might vary by as much as, say 10% - 20% between decadal periods or between major geographical regions. The variation among individuals within our samples encompassed a range equivalent to at least a factor of 5 (e.g., Fig. 5), so clearly there is the potential for significant variation among time periods or regions. To test for such variation would require preparation and analysis of 100 – 200 teeth from each population or time period.

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Table 1. Fractional ages used in age estimates for Alaska harbor seal teeth based on assumed birth in June.

June	Approximate birth
July	0.1
August	0.2
September	0.3
October	0.4
November	0.45
December	0.5
January	0.6
February	0.7
March	0.8
April	0.9
May	0.95

Table 2. Growth layer measurements (mm) for canine teeth from Southeast Alaska and Prince William Sound. Average growth layer widths, counts of measurements taken, and standard errors are shown for anterior and posterior margins of each canine tooth section.

		Southeast			Prince William Sound		
		Average	n	SE	Average	n	SE
Anterior	Fetal dentine	.452	45	.018	.437	9	.025
	Y-1	.561	43	.023	.563	11	.034
	Y-2	.563	31	.020	.567	7	.054
Posterior	Fetal dentine	.392	44	.013	.405	14	.017
	Y-1	.617	39	.032	.669	11	.043
	Y-2	.608	31	.028	.694	8	.049

Table 3. Results of *t*-test comparing Southeast Alaska and Prince William Sound regions.

Layer	<i>t</i> -value	Degrees of freedom	<i>P</i> -value
Anterior Fetal dentine	-.3551	52	.7239
Posterior Fetal dentine	.5317	56	.5969
Anterior Dentine Y-1	.0413	52	.9672
Anterior Dentine Y-2	.0814	36	.9355
Posterior Dentine Y-1	.8065	48	.4239
Posterior Dentine Y-2	1.4161	37	.1651

Table 4. Sample sizes required for 90% confidence of detecting specific differences of 10% - 50% between mean layer widths, using statistical tests at $\alpha = 0.05$.

Layer	10%	20%	30%	50%
Anterior Fetal dentine	141	36	17	7
Posterior Fetal dentine	87	23	11	5
Anterior Dentine Y-1	138	36	17	7
Anterior Dentine Y-2	93	24	12	5
Posterior Dentine Y-1	191	49	23	9
Posterior Dentine Y-2	127	33	15	7

Figure 1. Crown of tooth. Fetal dentine measurements were taken where enamel ends.

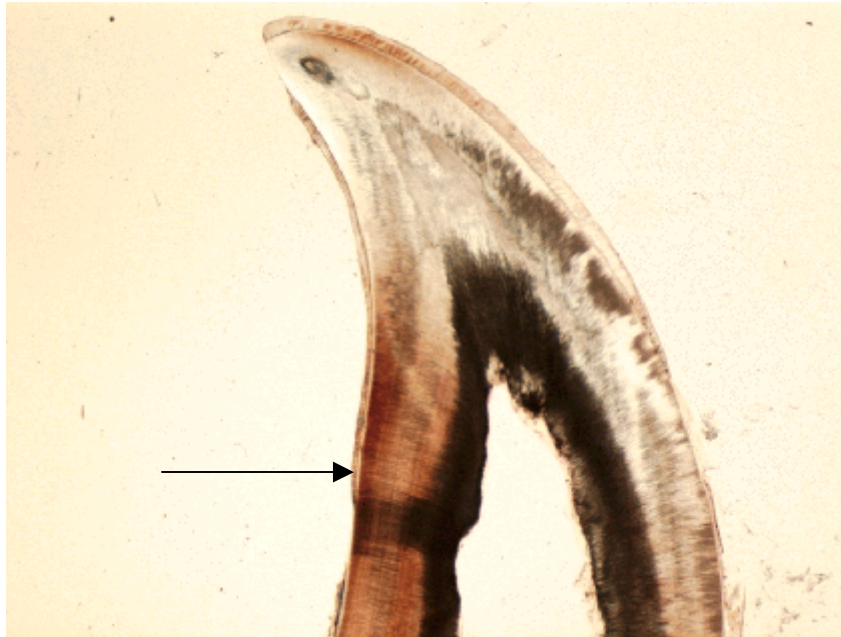


Figure 2. Fetal dentine-cementum overlap where annual dentine measurements were taken.

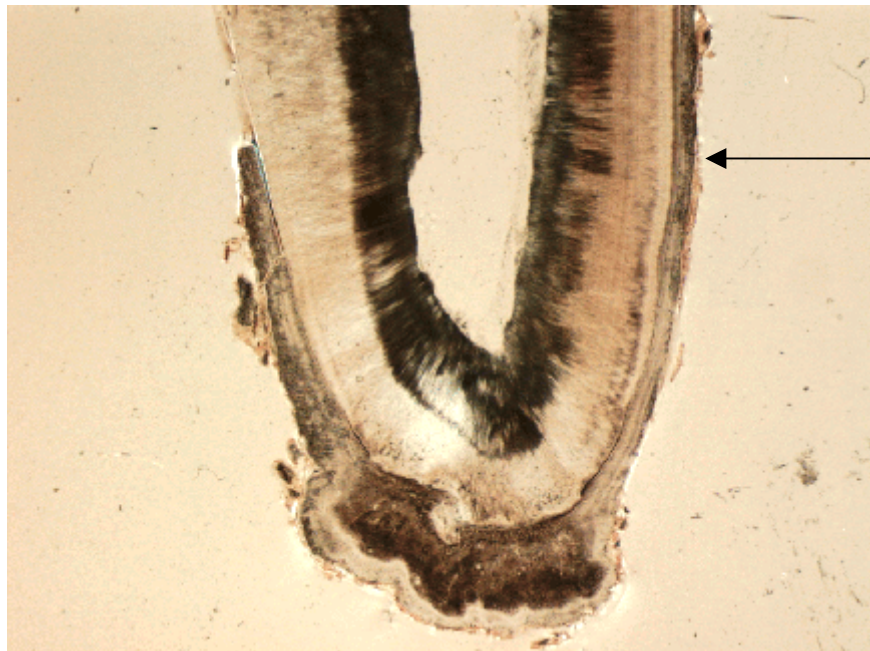


Figure 3. Relationship between ages from 50 canine and corresponding post canine teeth. Data are from two independent readers. Solid line represents a 1:1 correspondence of two sets of estimates.

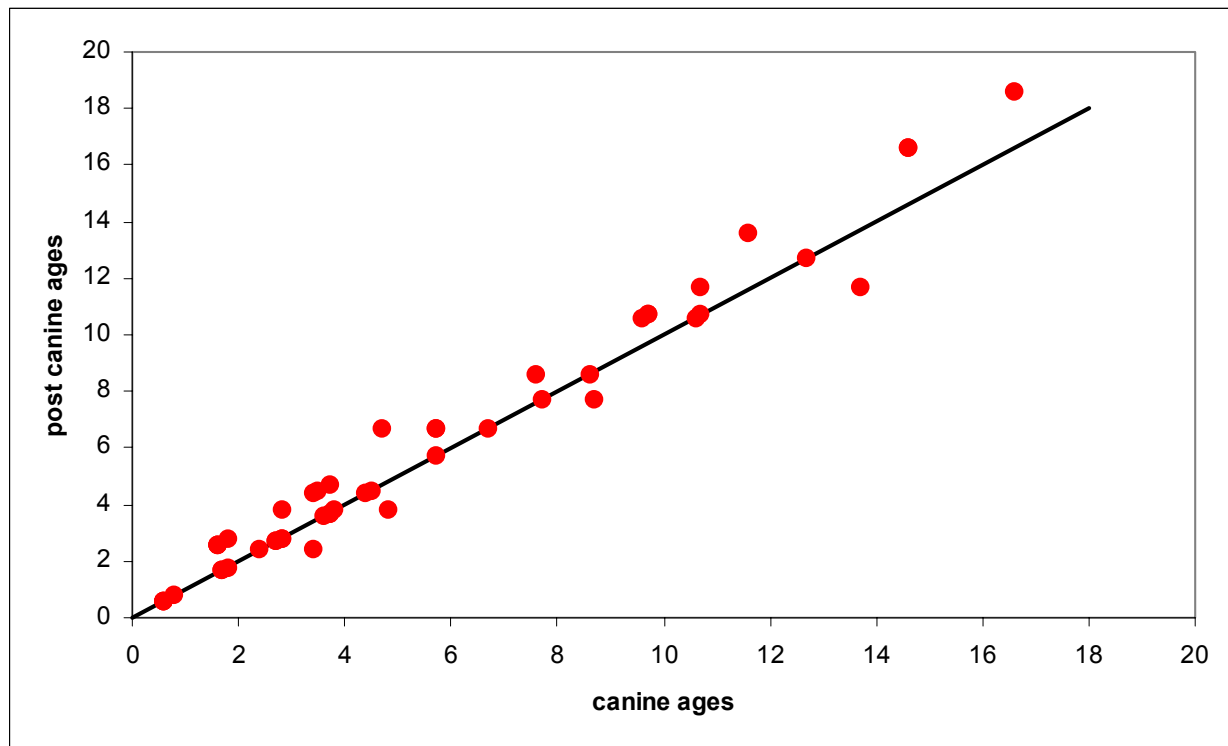


Figure 4. Age distribution for 72 harbor seal samples by region (PWS n = 15, Southeast n = 57).

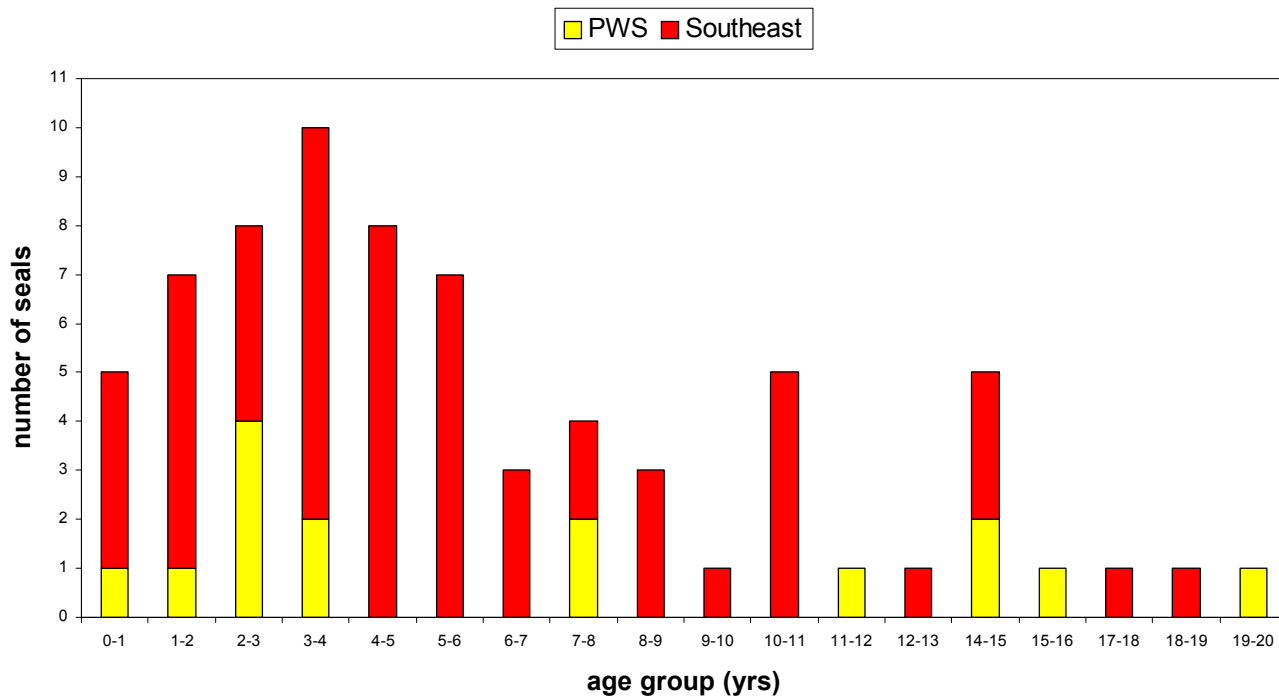


Figure 5. Plot of dentine growth in year one on posterior side of the tooth vs. dentine growth in year one on the anterior side of the tooth. All dentine measurements are in mm.

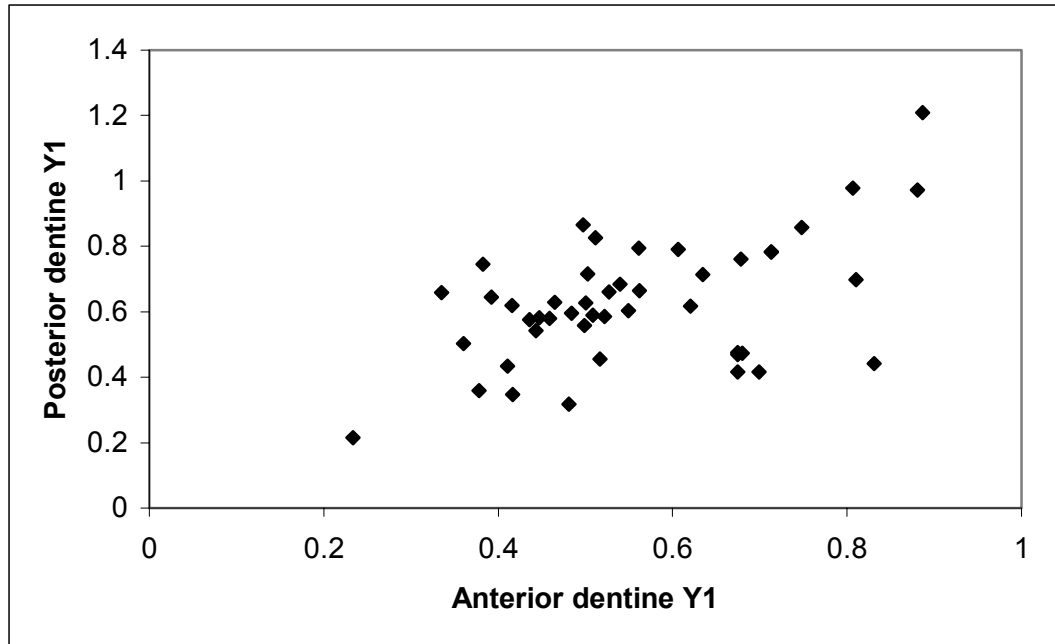


Figure 6. Plot of dentine growth (mm) in first and second year on anterior side of tooth only.

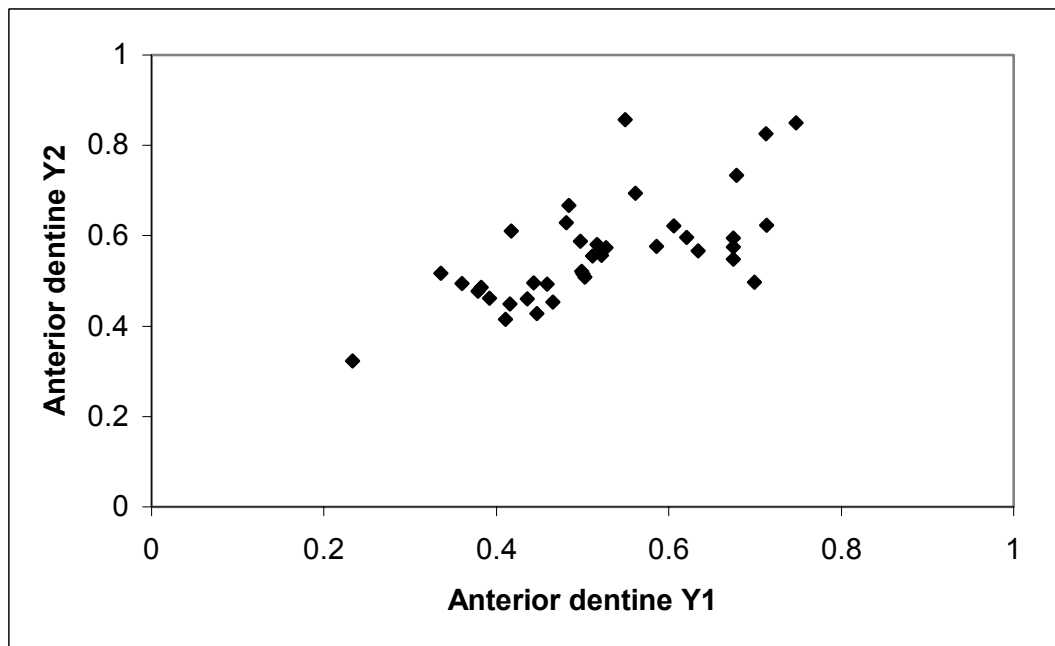


Figure 7. Plot of cohort year vs. first year of dentine growth on the posterior side of the tooth, measured in mm.

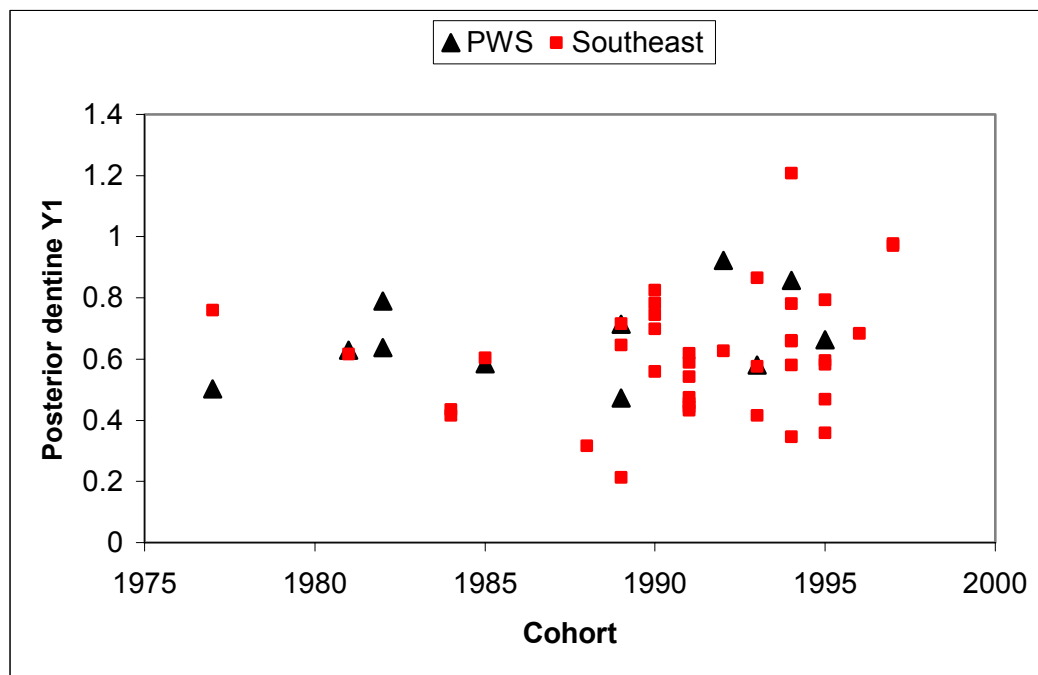


Figure 8. Plot of cohort year vs. first year of dentine growth on the posterior side of the tooth, scaled by dividing by the corresponding fetal dentine width.

